NASA Collaborative Agreement

NCC-2-127 Final Technical Report

March 1, 1981 - December 31, 1992

Effects of CSF Hormones and Ionic Composition on Salt/Water Metabolism

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Introduction

This collaborative agreement between Drs. Severs and Keil began in 1981, arising from a continuing interest in the issue of what, exactly, are the consequences of headward fluid shifts during manned spaceflight. Such shifts were recognized early by both U.S. and Soviet scientists because of signs and symptoms referable to the head. Some of these include disturbed vision, puffiness in the face and peri-orbital areas, headache, vestibular dysfunction and distended jugular veins.

We posited that the fluid shift had an (a) immediate effect on the brain and (b) a long-term action requiring a neural interpretation of the flight environment. This would re-adjust both efferent neural as well as hormonal mechanisms to sustain cardiovascular and fluid/electrolyte balance consonent with survival in microgravity.

This report summarizes work along these lines. Dr. Severs was primarily responsible for physiological, and Dr.Keil the biochemical and analytical aspects of the experiments. Historically, the research began under NASA grant NSG2122 to Dr. Severs but was converted to a cooperative agreement to better reflect the fact that both the research and publications were, indeed, collaborative. For continuity, this report includes an appendix that lists, separately, prior NSG2122 publications and NCC-2-127 sponsored research. Older NSG2122 work is not discussed; only a synopsis of some of the main NCC-2-127 research is

presented. Since the main research findings are published in the open literature, this synopsis is unreferenced, except for numbers that refer readers to the NCC-2-127 publications list for detailed information about the sponsored research.

Angiotensin and Vasopressin Actions in the CNS

The transition from the older NSG2122 grant to the present (NCC-2-127) cooperative agreement continued our original work on the concept that the peptide, angiotensin, altered blood pressure and hydration by central nervous system mechanisms. It is important to note that alterations in the renin-angiotensin-aldosterone system were recognized early in human spaceflight experience. However, in the mid 1970's, angiotensin was considered to act solely in the periphery. Neural actions were considered **not** to occur in normal physiology because the peptide should not cross the blood-brain-barrier.

For purposes of clarity, it should be stated that it is now known that all components of an **independent** renin-angiotensin system are present in the brain. The mRNA's for renin and it's substrate have been isolated from brain, and two receptor subtypes have been identified. Much of the biology evoked by angiotensin in the brain, like the blood-borne peptide, involves control of blood pressure and salt/water balance. Thus, it is apparent that **both** the brain and kidney renin-angiotensin systems must work in an integrated manner to achieve physiological homeostasis.

Since early flight biomedical data documented that the peripheral system was altered, it was necessary to evaluate brain angiotensin activity to see how it would adjust. A review of central vs peripheral angiotensin biology may be found in publication [3].

Similarly, in the case of vasopressin, early biomedical flight data provided evidence for diuresis and altered vasopressin actions. This peptide was considered to be acting solely on the kidney by way of the bloodstream. However, evidence that the peptide was endogenously present in multiple brain regions related to cardiovascular regulation and water balance necessitated an analysis of its central actions. Some specific questions and experiments supported by NCC-2-127 in the area of angiotensin and vasopressin activity in the CNS follows.

1. CSF Sodium Concentration as a Sensed Variable

Professor Bengt Andersson (Stockholm) had put forth the hypothesis that drinking behavior (water intake) and vasopressin (antidiuretic hormone) release was dependent on a rise in the sodium concentration of cerebrospinal fluid regardless of whether the afferent signal was induced by osmotic or volume mechanisms. Part of this hypothesis was based on the pronounced drinking behavior and vasopressin release evoked by small amounts of angiotensin in brain.

We developed a specific test of this hypothesis using conscious rabbits fitted with a sodium selective electrode in a lateral cerebral ventricle to provide continuous, on-

line recording of CSF [Na^+]. When exposed to either "volume" or "osmotic" stimuli, drinking and vasopressin release occurred without a rise in CSF [Na^+]. It remains possible that Na^+ flux in brain cells is a common signal, but **concentration** of the ion is not. An additional important observation made during these studies was that CSF renin activity increases after a hypertonic sodium chloride stimulus. This is opposite to plasma, where renin activity decreases. This was an important point in developing the concept that some central vs peripheral actions of angiotensin were reciprocal. Also, to the best of our knowledge, these were the first experiments to continuously monitor CSF [Na^+] in **conscious** animals. Reference # 1 provides details of method development and logistics.

2. Redundancy in Vasopressin Release

Literature in the early 1980's began to focus on the importance of angiotensin in the release of vasopressin. There was uniform agreement that small amounts of the peptide released vasopressin after instillation into cerebrospinal fluid. Like CSF [Na^+] referred to above, a central action of angiotensin was perceived as a link for both volume and osmotic release of the hormone. Specific experiments were designed to block angiotensin receptors in the brain before rehydrating **conscious** water-deprived rats. This form of dehydration activates both volume and osmotic control systems. Using protocols that clearly blocked the

activity of **exogenous** angiotensin, rats were able to re-hydrate properly when receptors for the peptide in the brain were blocked. Thus, mechanisms for rehydration have sufficient redundancy and angiotensin activity is not an absolute necessity. Intuitively, it makes physiological sense **not** to encode the response to dehydration to a single molecule (sic - angiotensin) without backup mechanisms. Our work provided documentation of this point (reference 7).

3. Mechanism of Angiotensin Natriuresis

One unexplained central effect of angiotensin that was the mechanism of natriuresis. Renal hemodynamics had been ruled out as an explanation. Two additional possible mechanisms were tested: mediation by renal nerves, or by a central action of vasopressin. These were evaluated in **conscious** rats given central injections of renin with or without concomittant vasopressin, and with or without intact renal nerves. Both of the possible explanations were ruled out (reference 2). Subsequently, later research determined that the most likely explanation was release of atrial natriuretic peptide.

4. Endogenous Opiate Interaction with Angiotensin

These studies were a continuance of work from past NSG2122 studies that showed that enkephalins and endorphins, given centrally, inhibited the cardiovascular and hydration responses to centrally administered angiotensin.
Experiments with **conscious** rats were performed using various combinations of enkephalin pretreatments followed by central

angiotensin injections. Collectively, this work revealed that enkephalin receptors reached from the third cerebroventricle inhibited the angiotensin-induced rise in blood pressure, drinking behavior and vasopressin release. Opiate receptors reached from the fourth cerebroventricle were only capable of reducing drinking behavior. (See reference 10).

5. Vasopressin in the Subfornical Organ (SFO)

The SFO is clearly a site of action of angiotensin that is reached from the blood and evokes drinking behavior and vasopressin release. Also, the SFO normally contains vasopressin that is packaged in "Hering-like" bodies similar to the storage of vasopressin in the neural lobe of the pitutitary. We studied whether vasopressin concentration in the SFO changed after water deprivation or stimulation by exogenous renin in a manner similar to the posterior pituitary. The intent was to see if vasopressin, of SFO origin, transduced the signal provided by angiotensin or dehydration into water intake and renal water conservation. The data obtained revealed that water deprivation increased [vasopressin] in the nearby hippocampal-fornix area, but not in the SFO body. Thus, if the neuronal elements of SFO use vasopressin for signal transduction, its content is not depleted by prolonged stimulation in a manner similar to vasopressin in the posterior pituitary gland. Reference 15 provides detailed information about this research activity.

6. CSF Neurochemical Concentration vs Blood Pressure

Angiotensin and vasopressin, under appropriate experimental conditions, raise blood pressure by actions on the central nervous system. Altered concentrations of these peptides, as well as many other neurochemicals and ions, have been proposed as a potential etiologic factor in elevated blood pressure. An experiment was designed to assess whether or not elevated **concentration** of neurochemicals in general was associated with high blood pressure.

The specific experiment utilized 3 groups of **conscious** rats: bilateral renal arterial stenosis of 30 days duration (chronic); sham-operated control rats, and unoperated control rats. The rats underwent a 5 hr cerebroventricular infusion of either artificial cerebrospinal fluid or glass distilled water at a rate of 2 ul/min followed by a 2 hr recovery period. Blood pressure was recorded from an indwelling carotid catheter and water intake and water and salt excretion were measured.

Blood pressures of the sham-operated and unoperated control rats were similar and both were much lower than the hypertensive rats. Neither solvent affected blood pressure when infused into the brain at any time over the 5 hr experiment. On the other hand, water infusion into the brain resulted in a clear diuresis and loss of free water in all groups. Since the infusion of **small** volumes of water promoted free water clearance it is likely that CSF dilution occurred; however, this did not affect central control of

blood pressure in either normotensive rats or rats whose blood pressure was elevated by neurogenic mechanisms. Reference 4 describes the experiment in more detail.

7. Central Vasopressin and Peripheral Hydration

Various experiments reported in the literature during the 1970's suggested that vasopressin, acting centrally, altered drinking behavior and/or decreased peripheral actions (i.e., change free-water clearance). Earlier experiments (supported by NSG2122) revealed that acute central injections of the peptide or its antiserum did not acutely affect consummatory behavior or urine output. However, since the half-life of vasopressin in CSF is about 20 min, potential longer term effects were uncertain. A series of experiments was done in **conscious** rats where the peptide was infused centrally for 5 hours or 5 days. Consummatory behavior and water and electrolyte excretion were measured. Vasopressin was without effect even though some of the dose levels were sufficient to "spill over" into plasma. Interestingly, in these cases, high plasma vasopressin did not reduce water excretion; this finding was subsequently confirmed in the literature.

Thus, high titers of vasopressin in CSF do not appear to affect consummatory behavior or salt/water excretion. On the other hand, similar types of experiments with the peptide revealed other significant biological effects, including memory processing, thermoregulation and intracranial pressure (see below). Reference 5 provides an

overview of vasopressin actions on consummatory behavior and urine excretion.

8. Seizures Evoked by Central Vasopressin Injections

Periodical reports in the literature have described acute seizures in conscious rats receiving central injections of vasopressin. This was generally regarded as some sort of nonspecific toxic reaction, as it occurred unpredictably and seemed to be indpendent of dose. We encountered this phenomenon, known as "barrel rotation" (BR) in the literature, during our studies on central effects of vasopressin dealing with salt/water balance and CSF pressure. An attempt was made to define this unique motor abnormality characterized by progressive ataxia, body swaying, lying on one side with spastic limb abduction and then rotation about the long axis of the body (BR).

A series of experiments was done where vasopressin was injected intracerebroventricularly to **conscious** rats to characterize this phenomenon. References 21, 22 and 27 should be consulted for full technical details. Here, a summary of major scientific points that emerged is presented.

First, in the dose range of 100 ng - 1 ug, the peptide produced an incidence of BR of about 50%. Latency analysis of this all-or-none event revealed a non-normal distribution. Hazard function evaluation indicated two separate underlying processes. One of these was eliminated in paradigms where rats could not use visual/vestibular

mechanisms to compensate for the potential seizure. Also, a process of "sensitization" was noted. When rats were retested with a smaller dose of vasopressin 2 days after the initial exposure, 80% of the animals displayed BR; all of the rats that were originally susceptible responded after re-exposure, and the increased incidence involved recruitment of rats that were previously resistant to motor destabilization. Antiseizure drugs of widely diverse chemical classes all had some protective effect against vasopressin-induced BR. Studies of regional cerebral glucose utilization revealed modest increases or decreases in brain sites that include areas related to visual/vestibular systems.

Research on this subject was stopped when Dr. Keil was advised that exploring these novel findings was outside of the scope of the RTOP for salt/water balance. However, further research into this area, not supported by NASA, has led to good documentation that BR is an excellent model of centrally mediated vestibular dysfunction, with similarities to "space sickness" (see Pharmacological Reviews 41:53-90, 1989 for an analysis of this area of research).

Intracranial Pressure Control

This section highlights several experiments done on intracranial (cerebrospinal fluid) pressure control. Three principal reasons for studying this subject exisited at the time this cooperative agreement began. Firstly, with regard to the issue of cardiovascular and fluid/electrolyte changes

in spaceflight, we considered that thoracic fluid shifts might raise intracranial pressure and therefore, the brain itself might provide the sensory component for compensation. Secondly, information in the literature that indicated that vasopressin in the CNS was associated with intracranial pressure. Thirdly, our hydration studies with centrally administered angiotensin revealed that conscious rats equilibrated with hypotonic plasma and reduced body mass. If saline was provided as a drinking fluid, rats tended towards the same state but fluid drinking and turnover was greatly increased. From a behavioral point of view, water drinking after angiotensin resulted in a more rapid equilibrium compared to saline-drinking, which was almost a constant behavior. Although plasma was hypotonic, we could not distinguish whether the brain was compensating for a perceived hypertonic or a hypervolemic condition. If the latter were a viable option, a rise in CSF pressure (CSFp) could be anticipated before compensation. The experiments described below highlight some of our achievements in this area.

1. Acute Effects of Angiotensin and Vasopressin on CSFp.

The above comments prompted an assessment of whether angiotensin and vasopressin acted centrally to change CSFp. A method and protocol was established using **conscious** rats that received a cerebroventricular infusion of artificial CSF for 30 min, followed by a "switch" for 30 min to artificial CSF with or without a peptide addition. Blood

and CSF pressures were measured. The following main points emerged. First, blood and CSFp's were similar and stable in all groups during the control 30 min. During the second 30 min (experimental) interval, CSF and blood pressures remained stable with infusions of artificial CSF or vasopressin. However, angiotensin produced both a centrally mediated rise in blood pressure and also raised CSF pressure from about 10 to 16 cm H_2O. When the two peptides were infused simultaneously, the rise in CSFp did not occur. Thus, vasopressin did not affect CSFp of normal rats, but was capable of blocking the newly discovered angiotensin-stimulated increase. Reference 11 provides details and additional data. To our knowledge, this was the first demonstration that high angiotensin levels in CSF elevated intracranial pressure.

2. Peptide Effects on CSFp over a 5 Hour Interval

Because the experiment described above yielded positive results showing that angiotensin infusion into CSF acutely raised CSFp, it was necessary to define the time course of this effect. The experiments to be summarized here employed **conscious** rats prepared for continuous infusions and pressure recordings from a lateral cerebroventricle over a 5 hr period. The details of these experiments are in references 20 and 25. It is important here to note that the infusion rate was 2 ul/min which is 100% of the endogenous synthesis rate of CSF in rats. It is also important to note

that, in the absence of obstructions to CSF drainage, CSFp is essentially independent of the rate of volume input.

The main findings from these experiments are as follows. First, angiotensin raised CSFp within 15 min, which peaked by 60 min and remained elevated for the remainder of the experiment. An important new observation however, was that the artificial CSF, by itself, caused an exponential rise in CSFp after a 2 hr lag, so that CSFp of the "control" and angiotensin groups were equal by 4 hr. The solvent-induced rise cannot be explained by volume overload, because when vasopressin was contained in the infusate, the rise in CSFp was markedly blunted. Also, when the infusate contained the angiotensin receptor antagonist, sar^1-ile^8-angiotensin II, the solvent effect was also markedly reduced.

Collectively, the data suggested (a) that some normal component(s) of CSF was required for pressure regulation and was "washed out" by prolonged infusions, and (b) angiotensin and vasopressin accelerated and inhibited the solvent-induced rise, respectively. Here, it should be pointed out that information from the literature documented that CSF vasopressin concentrations are elevated in humans with elevated intracranial pressure, regardless of the clinical cause. This includes the disease, benign intracranial hypertension (pseudotumor cerebri), where CSFp is elevated with no known clinical reason. Based on the above information, we undertook experiments to define what is

"control" or "normal" CSFp, and the general mechanism that elevates CSFp after infusions of a balanced, isotonic sterile salt solution (i.e., artificial CSF).

3. 24 Hour CSFp Monitoring & the Solvent Effect

The series of experiments highlighted here deal with the issues stated immediately above. Details of method development and quantitative information are contained in publications 29, 32, 36, and 45.

A method was developed to continuously record CSFp from **conscious**, relatively unrestrained rats over a 24 hr period using a computer-based system that sampled pressure every second. One of the major technical problems that occurred was that the choroid plexus often formed a seal around the probe tip that occluded pressure recording. For this reason, many experiments used an initial infusion of 8 ul/min for 10 min, followed by a continuous maintenance infusion of 0.5 ul/min to keep choroidal tissue from sealing the probe. In some of the experiments, the volumes of the inital and maintenance infusions were divided in half and infused into each lateral ventricle separately. Here, we will summarize the results from 3 groups of rats: uninfused rats where technical recording of CSFp was spontaneously obtained over 24 hr without exogenous infusions, unilateral infused rats that received the initial and maintanance infusions into one lateral cerebroventricle, and bilaterally infused rats where the exogenous infusions were equally divided into both lateral cerebroventricles.

Uninfused rats show a circadian rhythm in CSFp; it begins to rise in the late afternoon and peaks at night. Overall, normal pressure varies about two-fold over a day. Pressure can be visually correlated with the activity of the rat. For example, when a rat rears, or chews solid food, etc., CSFp (relative to the transducer dome) rises. It is interesting to note that blood pressure and vasopressin concentration in CSF show a similar circadian rhythm. We constructed an operational definition of "normal" CSFp to compare uninfused rats with those that required an exogenous infusion of artificial CSF to keep the recording lines open. The integrated 24 hr CSFp and S.D.of each **uninfused** rat was defined as 100% $\pm$ S.D. No uninfused rat ever had a CSFp more than 2 x it's S.D. **and** maintained it at that level for 2 consecutive hours.

Rats that received infusions were the same as uninfused rats for 2 hr. At that time, an exponential rise in CSFp of the general form, $f(t) = A + B * (1 - e^{-t/c})$ occurred. In the unilateral group, a peak was reached at 4 hr, and CSFp remained "abnormally elevated" according to the above definition for the rest of the 24 hr experiment. CSFp reached a peak at about 8 hr in the bilateral group; their CSFp's were higher than the unilateral group and also met the definition of "abnormally elevated". All infusions abolished the circadian rhythm in CSFp observed in uninfused rats. Rats receiving bilateral infusions differed from those receiving unilateral infusions in that neurological

symptoms were observed in 5 of 13 animals of the former group vs 0 of 12 of the latter ($p < 0.05$). Also, bilaterally infused rats drank less water over the 24 hr period compared to unilaterally infused animals.

Thus, the following questions emerged. a/ Why did the pressure curves of unilaterally and bilaterally infused rats differ when the infused volume was the same? b/ Aside from the initial infusion to clear the choroid, the 0.5 ul/min maintenance infusion was only 25% of the endogenous CSF synthesis rate. What happened in the 2 hr lag period that caused infused rats to develop abnormally elevated CSFp for the remainder of the experiment?

Alterations in the basic method were made to permit bolus injections during cerebroventricular infusions and pressure recordings. Estimates of compliance (**C**) and resistance to outflow (**Ro**) could be calculated from the decay kinetics. The following major findings emerged from this aspect of the work.

a/ It was found that the initial 10 min infusion to move the choroid away from the recording tip was, by itself, a sufficient stimulus to evoke the delayed rise in CSFp. The infusion, in our hands, duplicates data in the literature, showing a moderate increase in CSFp during the infusion and rapid recovery when the pump is turned off. Even without a maintenance infusion, the exponential rise in CSFp between hrs 2 and 4 occurred.

b/ In unilaterally infused animals, Ro and C were unchanged from baseline values during the 2 hr lag period. However, when CSFp was elevated, Ro was increased and C decreased.

c/ A protocol was used to inhibit CSF synthesis in rats receiving the 10 min initial infusion. This blocked the rise in CSFp that normally occurred from hrs 2 to 4. However, the infusion still caused a rise in Ro and a decrease in C. Since the effect of infusion on CSFp and Ro could be dissociated, we proposed that the infusion exerts a primary effect on mechanisms controlling Ro. CSFp rises with time **only** if normal CSF synthesis continues. The model has been proposed as a new method of studying abnormally high CSFp in conscious, non-traumatized and relatively unrestrained rats (see reference 45).

4. Other Studies on CSFp Control

a/ **Conscious** rats were gently restrained and their CSFp's were continuously monitored during a 3 hr period. The first hour was in the level position, the second hour was in a -45^{o} position, and the third hour was a recovery period. The data obtained revealed that the overall CSFp during the head-down tilt position was essentially normal. Only a small, transient (5 min) effect occurred when the tilt was initiated. Thus, under these conditions, intracranial pressure can quickly compensate and does not reach pathological levels. (See reference 43).

b/ The vena cavae of rats were constricted about 50% as they enter the heart or were left loosely tied. CSFp of the animals was measured in the immediate post-operative period, 1 and 10 days later. Terminal plasma [Na^+], [K^+], [AVP] and hematocrit were measured, as well as drinking and urine output from days 8 - 10. The intent of this experiment was to try to simulate thoracic volume overload by reducing rate of venous return of blood from the upper body to the heart. Conceptually, this should also reduce the venous/CSF pressure gradient that is the basis for CSF drainage at the arachnoid villa.

The results of the experiments showed that the measures listed above were similar ($p > 0.05$) for the sham operated and the venous-constricted rats. Thus, like the head-down tilt experiment described above, this experiment also showed that intracranial pressure, as well as indices of salt/water balance, rapidly adjust to conditions where a sudden fluid shift to the thorax may be expected to occur. (See reference 42 for details of this research)

c. Angiotensin or artificial CSF was infused into **conscious** rats at a rate of 2 ul/min in a protocol similar to that described above for 5 hour experiments. In these specific experiments, bolus injections were superimposed so that C and Ro could be calculated. The main result was that angiotensin increased CSFp without a change in Ro. Since it is unlikely that the peptide could stimulate synthesis by a sufficient magnitude to rapidly raise CSFp, the only general

mechanism remaining would be a rise in vascular volume. We note here that, almost simultaneous with our work, a German group found that angiotensin, acting from receptors reached from CSF, markedly dilated pial arteries by a nitric oxide dependent mechanism. This provides an explanation for our discovery of the angiotensin-stimulated rise in CSFp. Our experiments and the German data appear in publication 44.

d. Anesthetized rhesus monkeys were prepared for intracranial pressure recordings during two simulations of microgravity: -6^{o} head-down tilt and water immersion. The basic results were that during the relatively brief stimuli, intracranial pressure rose significantly ($p < 0.05$) and recovered rapidly after the stimulus was removed. The reasons why anesthetized monkeys showed an acute response whereas simulations described above for conscious rats did not are unclear. However, it should be noted that the rise in intracranial pressure in monkeys was relatively modest and did not appear to be in a pathological range. The experiment is described in reference 47.

Research on the Subcommissural Organ

Research beginning in the early 1980's provided clear evidence that the brain contained receptors for mineralocorticoids, but there was no information about the biological output of mineralocorticoid actions in the brain. Historically, the subcommissural organ (SCO) was associated with aldosterone secretion from the adrenal cortices. Also, **flight data** revealed increases in aldosterone secretion. We

designed experiments to examine whether aldosterone exerted central effects that could help explain salt/water balance changes observed in flight. References 14 and 26 contain the original data.

The general strategy was to continuously infuse aldosterone or solvent locally into the SCO region of **conscious** rats for **six** days and monitor consummatory behavior and urinary volume and electrolyte excretion. Terminal plasma samples were also assayed for ions and hormones.

Collectively, the following main points were found.

1. Aldosterone produced a biological effect **opposite** to its classical activity as a blood-borne hormone. Whereas the classical function is to cause sodium retention by the kidneys, aldosterone acted in the general region of the SCO to **increase** sodium excretion from the body as well as the urinary Na^{+}/K^{+} ratio.

2. Contrary to what would have been predicted by classical feedback endocrinology, central administration of aldosterone did not affect the morphology of the adrenal cortex or its zones, but produced a clear reduction in the cross-sectional area of the adrenal medulla with no change in the density of chromaffin cells. Paradoxically, this central action of aldosterone was correlated with a **specific** increase in plasma epinephrine concentration at the end of the experiment. Norepinephrine and dopamine levels were unaffected. It is important to note that these effects were

more circumscribed than those affecting sodium excretion. The adrenal effects required high precision in the SCO cannula placement, whereas natriuresis occurred if cannulae were slightly rostral (but not caudal) and confluent with CSF of the pineal recess. However, the main pineal body was excluded as a site of central aldosterone action in these experiments.

Comparative studies were done by infusing aldosterone directly into a lateral cerebroventricle. Under these conditions, none of the effects evoked from the SCO area occurred. However, the ventricular route of administration revealed a clear reduction in consummatory behavior.

To the best of our knowledge, these studies were the first to document that brain mechanisms controlling salt/water balance and consummatory behavior were responsive to aldosterone, and clearly establish that the brain is a target organ of mineralocorticoids.

Research on the Eye

Simulations of headward fluid shifts such as head-down tilt in humans raises intraocular pressure, which gradually returns toward normal. There are many similarities between issues associated with pressure volume relationships and salt/water balance within the eye and brain. The tissues which secrete and drain CSF and aqueous humor are remarkably similar on a histological basis. Therefore, as our work on CSFp control was developing, we also evaluated whether

neuropeptides affected intra-ocular pressure (IOP). One publication has appeared (35) and a working draft of our physiological data is in manuscript form and available from Dr. Keil (NASA-Ames) at the time of submission of this report.

Here, we summarize the main biological findings. First, we attempted to determine whether 3 major peptides known to affect CSF pressure were present in ocular tissue. These were angiotensin (ANG), vasopressin (AVP) and atrial natriuretic peptide (ANP). There was diffuse immunostaining for AVP and ANG in the uvea and retina in rats and rabbits. ANP, however, was sharply localized in the inner and outer plexiform layers of the retina but could not be localized in anterior structures of the eye.

All three peptides were identified in both the ciliary body/process and retina by sensitive radioimmunoassays. ANG, AVP and ANP (anterior eye only) may be stored in a manner unrecognized when the same antibodies were used for the immunohistochemical analysis. Perfusion of eyes with transcardiac phosphate buffered saline before collecting tissue for radioimmunoassay did not affect the amount of ANP or AVP measured in eye structures. This indicates that they did not originate from blood contamination and were not easily washed out of the eye. On the other hand, perfusion completely washed ANG out of the eye. This, and other published data lead to the conclusion that endogenous angiotensin in the eye is made and used locally. In the

case of all 3 peptides, the content in the ciliary body/process greatly exceeded the content in the retina on a wet weight basis. These experiments clearly established that the 3 neuropeptides are endogenously present in the eye. Since they are regulators of blood pressure and salt/water balance, as well as influence CSF pressure, analysis of their physiological effects in the eye was undertaken.

The physiological experiments wee done by direct pressure recording from the anterior chamber of the eye of anesthetized rats. Potential peptide effects on IOP were evaluated by 3 routes of administration (IVT, i.v., and direct infusion into the anterior chamber). Under the conditions used, central injections of active doses of the 3 peptides did not alter IOP. Equipressor doses of i.v. ANG raised, whereas AVP decreased IOP. The direct infusion studies revealed a novel finding. Similar to our studies with CSFp, low volume infusions into the anterior chamber of solvent (artificial CSF) raised IOP after a lag of 20 min. Co-infused ANG or ANP reduced this solvent effect, but AVP was markedly inhibitory. None of the peptides had an acute effect on **baseline** IOP when directly infused. The action of AVP was independent of aqueous humor formation, because it occurred in rats pretreated with acetazolamide. The AVP effect was reversed by a V1-type receptor antagonist. Superimposing bolus injections to determine resistance and compliance estimates within the eye showed that AVP produced

an acute increase in intraocular compliance. These novel data suggest that baseline IOP may be maintained by endogenous neuropeptides. Indeed, current literature is available indicating that blockade of angiotensin activity may lower IOP in animals and humans. Our data show that development of V1 receptor antagonists capable of reaching sites accessible from the anterior chamber may also have salutory effects on **abnormally high** IOP. Currently, since the co-operative agreement is terminated, we are completing our analysis of the data base and are modeling the pressure/volume dynamics of the eye and vasopressin-induced alterations.

We speculate that routine monitoring of IOP of astronauts during flight (a benign procedure) may be a useful indicator of potential changes in CSFp. We posit that there will be an initial rise, and that the rate of recovery of IOP during flight may help predict how well astronauts are adapting to the space environment, especially during long missions.

APPENDIX I

LIST OF PUBLICATIONS SUPPORTED BY NASA NCC-2-127

Walter B. Severs, Ph.D., Principal Investigator

1. Kapsha, J.M., Keil, L.C., and Severs, W.B. [Na^+] of lateral ventricular cerebral fluid in conscious rabbits before and after osmotic and hypovolemic stimuli. Exp. Neurol. 75:332-346 (1982).

2. Severs, W.B., Summy-Long, J.Y., and Keil, L.C. Contribution of vasopressin and renal nerves to the natriuresis evoked by centrally administered renin or angiotensin. In: The Renin Angiotensin System in the Brain. D. Ganten, M. Printz, M. Phillips and B. Scholkens, eds., Springer-Verlag, New York, pp. 324-334 (1982).

3. Severs, W.B., Summy-Long, J.Y., and Keil, L.C. The brain renin-angiotensin system. Drug Devel. Res. 2:231-239 (1982).

4. Maggio, W.W., Barbella, Y.R., Keil, L.C., and Severs, W.B. Effect of CSF dilution on blood pressure of renal hypertensive rats. Pharmacology 25:222-226 (1982).

5. Jerome, M.L., Barbella, Y.R., Wurpel, J., Keil, L.C., and Severs, W.B. Eating, drinking and urine output after prolonged cerebroventricular vasopressin infusions in rats. Pharmacology 26:79-84 (1983).

6. Wurpel, J.N.D., Dundore, R.L., Barbella, Y., Keil, L.C., and Severs, W.B. Barrel-rolling after intracerebroventricular arginine vasopressin. Fed. Proc. 42:363 (1983), Abstract.

7. Keil, L.C., Barbella, Y.R., Dundore, R.L., Wurpel, J.N.D., and Severs, W.B. Vasopressin release induced by water deprivation: effects of centrally administered saralasin. Neuroendocrinology 37:401-406 (1983).

8. Wurpel, J.N.D., Barbella, Y.R., Keil, L.C., and Severs, W.B. CSF pressure during intracerebroventricular angiotensin and vasopressin. Pharmacologist 25:156 (1983), Abstract.

9. Dundore, R.L., Keil, L.C., Barbella, Y.R., Wurpel, J.N.D., and Severs, W.B. Saralasin effects on vasopressin release in water deprived rats. Pharmacologist 25:156 (1983), Abstract.

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APPENDIX II

List of Publications Supported by NASA NSG2122

Walter B. Severs, Ph.D., Principal Investigator

1. Summy-Long, J. Crawford, I.L., and Severs, W.B. Effects of subfornical organ extracts on salt-water balance in the rat. Brain Res. 113:499-516 (1976).

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